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CASTANOSPERMINE—A PLANT GROWTH REGULATOR

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Abstract—Castanospermine, 1,6,7,8-tetrahydroxyoctahydroindolizine, has been shown to be a potent dicot phytotoxin. The effective concentration to inhibit root length elongation of dicot roots by 50% is 300 ppb, while the effective concentration against monocot roots is 200 ppm, i.e., 10^3 times less effective. In contrast swainsonine, 1,2,8-trihydroxyoctahydroindolizidine, is ineffective as an inhibitor of root length elongation.

Key Words—Castanospermine, phytotoxin, glucosidase inhibitor, swainsonine.

INTRODUCTION

The indolizidine alkaloid, castanospermine (Scheme 1, **1**), 1,6,7,8-tetrahydroxyoctahydroindolizine, isolated from the seeds of the Australian legume *Castanospermum australe* (Hohenschutz et al., 1981), is a potent inhibitor of fibroblast α- and β-glucosidases and almond emulsion β-glucosidase (Saul et al., 1983). In addition, it inhibits the processing of the oligosaccharide portion of the influenza viral hemagglutinin (Pan et al., 1983) as well as the processing of plant *N*-linked oligosaccharides in soybean cells (Hori et al., 1984). Nojirimycin (**2**) (Inouye et al., 1968), an antibiotic structurally related to castanospermine, is a potent inhibitor of α- and β-glucosidases and amylases (Reese and Parrish, 1971) and of plant auxin-induced cell extension, while castanospermine itself is an effective inhibitor of cell-wall associated β-glucosidase from corn roots (Nagahashi et al., 1986). In view of these results, we felt that it would be of interest to determine whether castanospermine was phytotoxic by a test system previously found successful to delineate phytotoxicity (Stevens,

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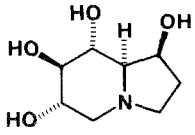

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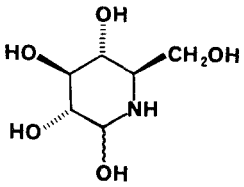

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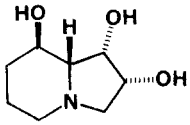

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SCHEME 1.

1986). The involvement of glucosidases in cell-wall degradation and synthesis (Inouhe, 1984; Koyama et al., 1983; Nevins, 1970, 1975; Nishitani and Masuda, 1983; Sakurai and Masuda, 1977) further suggests the potential utilization of castanospermine as a probe into the biochemical mechanism of cell growth.

METHODS AND MATERIALS

Isolation of Castanospermine. Castanospermine (1,6,7,8-tetrahydroxyoctahydroindolizine), mp 218–220°C, was obtained (0.3% yield) from mature seeds of *Castanospermum australe* by water extraction and purified by ion-exchange chromatography on Dowex 50W-X8 (Hohenschutz et al., 1981).

Isolation of Swainsonine. Swainsonine (1,2,8-trihydroxyoctahydroindolizidine, mp 144–146, was obtained from *Astragalus lentiginosus* as previously described (Molyneux and James, 1982).

Bioassay. The effect of castanospermine on root length elongation of lettuce (*Lactuca sativa*, black-seeded Simpson), alfalfa (*Medicago sativa*), barnyard grass (*Echinochloa crusgalli*), and red millet (*Panicum miliaceum*) was tested at 0, 10, 20, 40, 80, 100, 200, 400, 800, and 1000 ppb (or ppm in the case of monocots) in 40 ml of 0.5% agar (Bacto-Agar, Difco Laboratories, Detroit, Michigan) in 9-cm Petri dishes. All test seeds were first germinated in Petri dishes on 0.5% agar in a growth chamber (58°F, 8-hr nights; 68°F, 16-hr days). Twelve germinated seedlings were transferred to Petri dishes containing castanospermine and incubated in the dark (21–23°C, 45–72 hr). The lengths of the roots of the 12 seedlings were measured to the nearest millimeter and the

highest and lowest values were discarded. The remaining 10 root length measurements were subjected to statistical analyses. Two replicates were run for each test species. A parallel series of experiments was run using swainsonine (**3**) rather than castanospermine (**1**).

Data Analysis. Seedling growth assay results were statistically analyzed separately at the Washington, D.C., Computing Center facilities and Statistical Analysis System (SAS Institute, Inc., Cary, North Carolina, 1982). The transformed data were subjected to Cochran's test for homogeneity of variances of all treatments and the least significant difference (LSD) test for differences between all treatment means.

RESULTS

The effect of castanospermine (**1**) on lettuce root (*Lactuca sativa*) elongation is shown in Figure 1. At 300 ppb (1.6×10^{-6} M), castanospermine inhibits root elongation by approximately 50%. Addition of 10^{-10} M indole acetic acid (IAA), a concentration determined to promote root elongation, did not nullify the inhibiting effects of castanospermine. Although many plant growth inhibitors exhibit "auxin-like" characteristics, i.e., they display a growth-promoting activity at lower concentrations, castanospermine showed no such activity down to 10^{-10} M. The effects of castanospermine on alfalfa (*Medicago sativa*) was similar to that of lettuce, i.e., a 50% inhibition of root length elongation was observed at 250 ppb. Again the addition of IAA at a concentra-

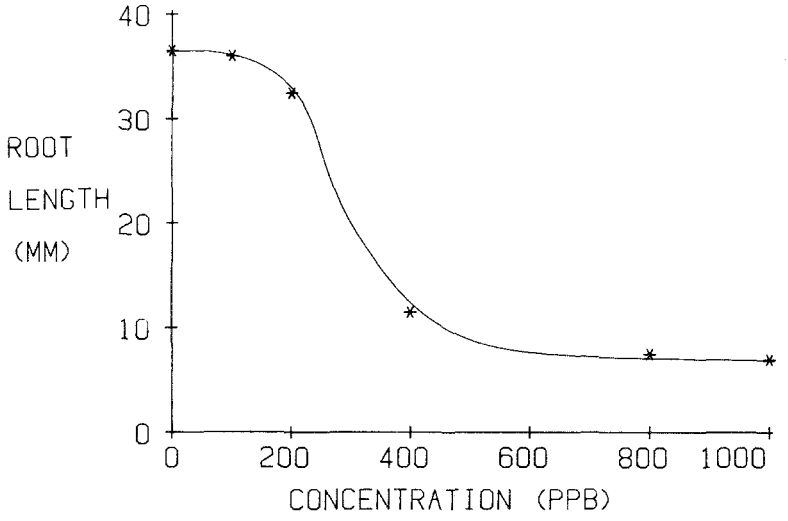

FIG. 1. Effect of castanospermine (**1**) on root growth of lettuce.

TABLE 1. EFFECT OF CASTANOSPERMINE ON ROOT LENGTH OF RED MILLET

Conc. (ppm)	Mean root length (mm)[a]	N^b
control	30.2 a	14
1	27.9 a	15
2	31.9 b	14
4	33.3 b	15
10	29.6 b	15
20	26.7 b	15
40	20.8 c	15
100	14.9 d	15
200	10.0 e	15
400	8.7 e	15
1000	7.2 f	15

[a] Means associated with a given test seedling with different letters are significantly different at the $\alpha = 0.05$ level according to Duncan's multiple-range test performed on transformed values.
[b] Number of roots measured.

tion that promotes elongation did not nullify the inhibitory effects of castanospermine.

Monocotyledons, such as red millet (*Panicum miliaceum*) and barnyard grass (*Echinochloa crusgalli*), were also inhibited by castanospermine (Table 1) but at a much higher concentration than for the dicots. For instance, red millet root elongation was inhibited 50% at 200 ppm (8×10^{-4} M) castanospermine. Barnyard grass gave similar results (not shown). Again, the addition of IAA had no apparent effect on nullifying the effects of castanospermine on these grasses.

DISCUSSION

The differential effect of castanospermine on the root length elongation (or inhibition) of dicots and monocots may well reflect the preferential inhibition of enzymes present in living plant tissue needed to degrade primary cell-wall structures. Evidence is substantial (Inouhe et al., 1984; Koyama et al., 1983; Nevins, 1970, 1975; Nishitani and Masuda, 1983; Sakurai and Masuda, 1977) that the auxin-induced elongation of cells is correlated with the degradation or depolymerization of cell-wall xyloglucans which lead to cell wall loosening and therefore cell extension growth. Studies with *Vigna angularis* (azuki bean) (Nishitani and Masuda, 1983) offer evidence that IAA-induced changes in the xyloglucans are related to the degree of cell-wall loosening rather than cell extension growth.

Substantial structural differences exist in the xyloglucans of monocots and dicots (Kato and Matsuda, 1976; Kato et al., 1981; Masuda, 1980), which may account for the differences in the inhibition of the root elongation of these plants by castanospermine. In addition, monocots have β-1,3- and 1,4-linked glucans, hemicelluloses not generally found in dicots. Furthermore, monocots have substantial amounts of glucuronoarabinoxylan (Burke et al., 1974), another hemicellulosic component. These differences could readily account for the observed differences in the effectiveness of castanospermine since it is an effective inhibitor of glucosidases (Saul et al., 1983), enzymes necessary for the degradation of dicot hemicelluloses. Since the structurally related compound, nojirimycin, is a much weaker inhibitor (by a factor of 1000) of glucanases vs. glucosidases (Reese and Parrish, 1971), enzymes necessary for the degradation of monocot hemicelluloses, castanospermine would be expected to have similar inhibitory activity against glucanases. Therefore, dicots would be expected to be inhibited more effectively than monocots by castanospermine. The specific enzyme inhibitory activity of castanospermine thus would interfere with primary cell-wall degradation, a step necessary to cell-wall elongation. Castanospermine does inhibit glucanases; however, a much higher concentration is required than for glucosidases. The effective concentration of castanospermine to inhibit root length elongation of monocots is about 900 times higher than that for dicots, approximately the difference in effectiveness of nojirimycin toward glucanases and glucosidases (Reese and Parrish, 1971).

Analogous experiments with swainsonine (**3**), a known inhibitor of α-mannosidase (Colegate et al., 1979; Kang and Elbein, 1983) but having little effect on glucosidases, showed no effect on the root length elongation or inhibition of either monocots or dicots. These data are consistent with the fact that the cell wall contains little mannose, hence α-mannosidase is not involved in the degradation of cell walls leading to cell elongation.

It has been observed that mannitol inhibits auxin-induced cell elongation by osomosis (Nevins, 1975). Since castanospermine may be considered to have many structural features analogous to carbohydrates, the possibility exists that its effect on monocots is a result of the same phenomenom as mannitol. Mannitol is effective at about 250 mM which is about 10^3 less active than castanospermine. Hence, it seems unlikely that castanospermine is decreasing the turgor of the cells by osmosis, thus interferring with growth.

A closer look at the specific enzymes inhibited by castanospermine and the relative amounts necessary to induce inhibition will lead to a better understanding of cell growth of both monocots and dicots. Castanospermine thus may be used as a probe to precisely define and understand the various polymers that make up the primary cell wall and the mechanisms of depolymerization and/or polymerization leading to cell growth.

We have observed that in order for *C. australe* seeds to germinate they

must be irrigated with water for a considerable period of time. It appears possible that the castanospermine present may therefore be self-inhibitory to germination and growth of the seeds, perhaps until rainfall is sufficient to support further growth. In addition to its effect upon root elongation, castanospermine is a powerful antifeedant to the pea aphid (Dreyer et al., 1985) and has been shown to differentially inhibit disaccharide-hydrolyzing enzymes in a broad taxonomic spectrum of insects (Campbell et al., 1987). Castanospermine may therefore serve a multiple ecological role in conferring an advantage to *C. australe* in competition with other plants and insect predators.

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